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NUTRITION REVIEWS

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NUTRITION REVIEWS

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EXPERIMENTS AND THE STATISTICIAN

Not long ago, optimistic experimenters were wont to bring statisticians masses of data with requests for analyses, only to be told that if they had included this or the other item of information, an answer might have been forthcoming. This was hardly fair to the experimenter, who thereupon told the statistician that, if he knew so much, he might better tell beforehand what information should be collected, or else. This, again, was not entirely fair to the statistician, who knows much less than the investigator about what may be expected from a proposed experiment before it is done. This note attempts to point the way out of the dilemma.

Far more mistakes in the statistical field are made by poor original planning of experiments, and above all by misinterpretation of results, than by arithmetic errors or misuse of statistical technics. The mathematical methods which need be employed in most biologic investigations are simple and lend themselves to only minor distortions. The fallacies encountered fall mainly into two categories: (a) failure to ask the question clearly and in a manner subject to answer, which we may call "no question"; (b) the assumption that the answer applies to one question whereas in truth, it refers to an entirely different one which has perhaps not even been consciously asked. This might be called "wrong question."

The first crucial question in any experiment is, generally, "Is there any relation between the two factors I am investigating?" The second, which immediately follows in case of a positive answer, is, "How close is the relation and what is its form?" Let us assume that the experimenter wants to know whether Factor X in the diet changes the weight curve of animals. This must have an answer before it is worth while to ask the second part of the question—"How much and under what conditions?"

In answering questions we make frequent use of "significance tests" which we may here use to illustrate the argument. The significance test has a legitimate place in science but, like most items of technic, must be understood before it can be well used. There are whole families of significance tests and there may be reasons for preferring one or another under different conditions. All of them are designed to answer a question if it is put to them in the proper way, and all good ones do so with only minor variations between them.

Strictly speaking, the question asked by us comes down to: "Does the 'population' of all animals who receive, have received, or ever will receive Factor X differ from the 'population' of all who do not? Secondly, if so, how much?" Our difficulties arise from the fact that we can never examine more than a sample, usually small, from either of these populations. The first sample is the experimental group, the second the control. Now the animals in neither population are uniform. Some are young, some old, and they differ in numerous other ways, any of which could conceivably influence either the effect of Factor X or the growth curve, or both. There is no guarantee that our experimental group may not accidentally become heavily loaded in one direction while the control group accidentally gets an opposite bias, thus producing a difference in the outcome of the two groups which could be interpreted as the effect of the administration of Factor X.

There is, on the contrary, every expectation that two samples selected from even the same population will differ from each other by accident. The more diverse the parent population, the greater the expected devia-

tion of the samples. However, the greater the number of individuals in the two samples, the less their expected deviation. It is precisely the function of the significance test to measure the probability of deviations of different sizes between samples from the same population. If the observed deviation is one which is unlikely to occur by chance, it is assumed that the two samples represent distinct populations and that the difference is "real" or, in this case, that there is a difference between the animals which receive Factor X and those which do not. In the contrary case, we decide that the two samples could easily have come from the same population.

The results are frequently stated in the form of a value of "P": the probability that the observed difference could arise between samples from the same population. If P is small (about 0.05), the difference is called "significant." If it is as small as 0.01 or smaller (that is, the difference would occur accidentally once in a hundred tries or less), it is usually "highly significant." Obviously, just what is called "significant" is a sort of fielder's choice, though nobody would quarrel with the principle of accepting as "real" a difference which could only rarely be due to accident.

Now notice that when P is not small we have merely said that the difference could easily have been accidental, not that it necessarily must be. A real difference may be missed by taking samples so small that even a large divergence could easily be accidental. Conversely, large samples may demonstrate as "real," differences so small that they are of no conceivable importance. By "significant," then, we mean "probably real" and not by any means "certainly important."

Now, in order to avoid the second fallacy of "wrong question," let us remember that we asked merely, "Is my observed difference probably actual?" We have not asked what is responsible for the difference and we must guard against accepting a positive result as the answer to an entirely different question, viz., "Does Factor X produce a difference?" The answer to the latter question demands much additional information, not all of which may be at the statistician's disposal.

For, while Factor X may produce a difference, so may a poorly selected control, or a biased observer, or any one or more of a multitude of other causes, some of which may be most difficult of detection. In the field of nutrition, and above all in that of human nutrition into which psychic factors may enter at any point, the selection of a completely balanced and unbiased experimental and control combination may be a difficult thing. Here the statistician should certainly be asked to help, for mutual protection, if he is to help later with the analysis.

Nor does the original plan determine the entire experiment, statistically or in any other way. Experimental procedures may vary considerably between beginning and end, as information accumulates. Changes may be made which are so obvious to the experimenter that they do not appear in the data. The statistician may entirely miss these, unless he has been closely associated with the entire course of the experiment. Yet some neglected minor factor at times makes the difference between a rational analysis and a ludicrous error.

The answer, then, is that only part of the statistician's value is at the end of the experiment, when he analyzes results. It is multiplied if he can assist in the planning of the original work, to see that statistical criteria are satisfied. He can be fully useful only if he is thoroughly conversant with all aspects of the experiment and its development, and for this he should be one of the staff associated with the work, or at least in frequent and intimate contact with it. This does not at all mean to imply that the experimenter himself should not have a thorough, even if narrow, acquaintance with the sort of quantitative thinking which has been called "common sense reduced to arithmetic."

HUGO MUENCH,
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NUTRITIONAL REQUIREMENTS

The Table of Recommended Dietary Allowances proposed by the Food and Nutrition Board of the National Research Council has served its purpose for four years as a guide in dietary planning. During this time there have been comments that the recommendations may have been set too high. Wilder (*Science* **101,** *285 (1945)*) has defended these standards and analyzed their misinterpretations and misuses. It is pointed out that the levels set were not arbitrary but were based on accumulated experiments on human subjects subsisting on diets of predetermined composition, on extensive animal experimentation, and with appreciation that such recommendations should accommodate those normal individuals whose requirements may exceed the average. The allowances were admittedly set to allow for a "factor of safety." It has been argued by some that the allowances should be based on minimal average requirements, but as Wilder states, such a figure assumes perfect distribution within a group. It is doubtful whether such a condition can be assumed in a given population group, and certainly a given individual cannot determine whether his particular needs are below or above average.

Much of the present disagreement among nutritionists has arisen from a failure to recognize that all physiologic attributes are characterized by variation. The mean or arithmetic average of a series of observations on these attributes, taken alone, "is of very limited value, for it can give no information regarding the variability with which the observations are scattered around itself" (Hill, "Principles of Medical Statistics," *Third Edition, p. 29, The Lancet Ltd., London (1942)*). Therefore, if dietary standards are based upon average figures, "we should know for every figure a distribution curve, so that we could have some idea of applying probability to a table of dietary allowances. Otherwise, when you are dealing in the mass, that is from a public health standpoint, it is difficult to know how to interpret many results" (Pett, *Proc. Res. Conference on the Relation of Nutrition to Public Health, p. 22, The Nutrition Foundation, New York (1943)*).

Pett has expanded this concept in a clear and convincing manner (*Canad. J. Pub. Health* **36,** *69 (1945)*). He has presented distribution curves for the requirements of calcium, thiamine, and iron based upon data in the literature. The variation in the adult human requirement for physiologic balance of calcium may be taken as an example of the argument: "Of 97 persons a balance between intake and output of calcium was achieved for 22 persons with less than 0.35 grams daily, while 54 persons were in balance with between 0.35 and 0.55 grams calcium daily, and three of the people needed over 0.70 grams daily. The mean or average, for this group, and the mode (i. e. the most common individual balance requirement found) are between 0.40 and 0.45 grams calcium daily." By contrast, the recommended allowance of the National Research Council is 0.8 g. As Pett concludes, it is obvious that "only a small fraction of a population group apparently needs this much, and that nearly half of the group could be in calcium balance with a calcium intake of only 50 per cent of our standard." Similar variations are indicated by the data for the other two factors discussed.

Thus, as Pett emphasizes, the finding that a high percentage of a population is eating less than 50 per cent of the recommended allowances of at least certain factors might not be an unexpected finding. Hence, it is illogical to speak of a given percentage of the populaion as suffering from a deficiency, however mild, on the basis of such evaluation of dietary data alone.

It is unfortunate that the Table of Allowances has been misinterpreted by certain groups. The foregoing discussion makes it clear that the figures cannot be rigorously

applied to the individual. The term "allowance" rather than "requirement" has been used to avoid precise conditions of minimum needs which may be affected by a variety of special conditions. It can be expected that the Food and Nutrition Board, as a scientific group, will with impartial judgment of new evidence, revise its estimates of dietary allowances if such is deemed necessary, in fact a revision has recently been made (*Nutrition Reviews* **3,** *287* (*1945*)).

EFFECTS OF OXALIC ACID IN THE DIET

It has been known for many years that a number of the green leafy vegetables contain rather extraordinary amounts of oxalic acid. For example, spinach, New Zealand spinach, Swiss chard, beet tops, lambs quarters, poke, purslane, and rhubarb leaves contain, on a dry weight basis, as much as 10 per cent oxalic acid (for the most part in the form of its salts). Traces of oxalic acid are also found in many other fruits and vegetables (Kohman, *J. Nutrition* **18,** *233* (*1939*)). These facts have attracted the attention of nutrition workers from time to time because of their possible bearing on the utilization of calcium.

Remington and Smith (*Science* **101,** *271* (*1945*)) report that groups of rats receiving in addition to other foods the equivalent of 25 g. of fresh spinach daily in the form of a commercially prepared powdered spinach (Spintrate) showed no evidences of calcium deficiency over a period of thirty-five days as measured by bone ash and body growth. The authors point out that this level of intake is equivalent to $2\frac{3}{4}$ pounds of fresh spinach for a human dietary of 2500 calories and conclude that "it would seem that spinach, even in such relatively enormous amounts as would never be taken by human beings, would not exert a deleterious effect on growth or on bone formation unless the customary diet of the individual were lacking in calcium."

Kohman (*Science* **101,** *610* (*1945*)) declares that the Remington and Smith report not only fails to clarify the problem, but gives the wrong impression of the established and published data on the subject. It has been determined by previous work, as Kohman points out, that oxalates in spinach have no effect on rat growth or bone if the diet contains adequate calcium to match stoichiometrically the oxalate in addition to the normal calcium requirement. However, it is also clear that when spinach, known to contain oxalate, is fed alone or in a mixed diet, calcium utilization is greatly decreased. For example, Fincke and Sherman (*J. Biol. Chem.* **110,** *421* (*1935*)) showed that when rats were fed diets containing 0.33 per cent calcium, half of which was supplied by milk and half by spinach, only 40 per cent of this calcium was deposited in the bones of the growing animals, whereas when the spinach was replaced by kale (an oxalate-free leafy green) the deposited calcium rose to 80 per cent. Kohman later reported similar findings and showed further that a diet which was satisfactory in calcium for growth could lead to injury by the addition of oxalate-containing leafy greens. However, if enough calcium were added to the defective diets they again became innocuous. Thus there seems to be no doubt that oxalates do interfere with efficient calcium utilization. Whether this interference leads to a deficiency depends upon the oxalate and calcium contents of the diet.

The loss of calcium by combination with oxalates does not seem to be a serious problem in the majority of American dietaries, since high oxalate-containing foods are not consumed regularly enough. However, there are probably instances in which such calcium loss is an important factor and,

therefore, the facts cited above should be kept in mind. In any event it is evident that before one can definitely arrive at a decision in a particular case, both the calcium and oxalate content of the diet must be considered. As Kohman points out, Remington and Smith do not give this requisite informotion but evidently the calcium of their diet was high enough so that in spite of the oxalate in the spinach, the animals were able to absorb adequate amounts of calcium. Many readers of the Remington and Smith article will be left with the impression that evidence has been presented that almost unlimited consumption of oxalate containing food (i.e., $2\frac{3}{4}$ pounds of spinach daily) would be without effect. The evidence presented does not warrant such a conclusion nor do the authors make such a general statement. It is interesting that the oxalates in $2\frac{3}{4}$ pounds of spinach would combine with 2 to 3 g. of calcium.

THE PROTECTIVE ACTION OF PROTEIN AGAINST TOXIC CHEMICALS

There is an increasing interest in the effect of diet on the toxicity of drugs and a mounting realization that proteins constitute an effective protective agent against a wide variety of toxic compounds. Diets containing adequate amounts of protein have been shown to exert a beneficial influence when administered to experimental animals exposed to chloroform, carbon tetrachloride, or benzene, or when administered to animals given toxic amounts of selenium, trinitrotoluene, arsphenamine, mapharsen, or atabrine. Several reviews concerning portions of this experimental work have appeared in this journal (*Nutrition Reviews* **1,** *425 (1943);* **2,** *207, 292, 340 (1944)*).

The interest in protein as a protective agent against liver injury from halogenated hydrocarbons derives from the investigations of Goldschmidt, Vars, and Ravdin (*J. Clin. Invest.* **18,** *277 (1939)*). Most earlier investigators had believed that protection of the liver was best afforded by a high carbohydrate diet. Goldschmidt *et al.* concluded from their experimental results with mice that the known deleterious effect of high fat diets was due to the resultant high concentration of lipid in the liver and that the protective action of high carbohydrate diets was due chiefly to their effect in reducing hepatic lipid. They recognized the lipotropic action of protein foods but concluded that the striking protective activity of adequate dietary protein resulted from a more direct mechanism. Miller, Ross, and Whipple (*Am J. Med. Sci.* **200,** *739 (1940)*) showed that methionine, or to a lesser extent cystine, gave almost complete protection against chloroform poisoning to the protein depleted dog. Miller *et al.* postulated that as far as the experiments with chloroform are concerned the protein depleted dog is one depleted of cystine and methionine, and that protein protects animals against the toxic effect of chloroform anesthesia by making available methionine and cystine. They postulated further that the sulfur containing amino acids act as direct detoxicants of chloroform. Miller and Whipple (*J. Exp. Med.* **76,** *421 (1942)*) later showed that methionine (or cystine plus choline) gave significant protection even when given three to four hours after chloroform anesthesia.

Heppel and co-workers (*J. Indust. Hyg. Toxicol.* **27,** *15 (1945); J. Pharmacol. Exp. Thérap.* **84,** *53 (1945)*) have added 1,2-dichloroethane to the list of halogenated hydrocarbons studied. Their results serve to focus attention on the dual role played by protein or rather by methionine in protecting against the toxic effects of this type of compound. Under certain experimental conditions methionine will exert its beneficial effect largely by virtue of its lipotropic activity; under other experimental condi-

tions its activity is of a different type, perhaps direct detoxication as postulated by the Rochester investigators.

In their earlier experiments, Heppel *et al.* employed a control diet of "normal" protein and fat content (approximately 10 and 25 per cent, respectively) and experimental diets which were, respectively, low in protein with normal fat content, low in protein and high in fat (with or without added choline or added choline and methionine), high in fat with normal protein content, and high in both protein and fat. Weanling rats were prepared on these various diets for periods ranging, in different experiments, from two to thirty-one days and were then exposed to 1,2-dichloroethane inhalation. The concentration of the hydrocarbon in the inhalation mixture was 400 or 1000 parts per million. In some of the experiments a paired feeding technic was employed. The rats receiving the low-protein, high-fat diet (containing 40 per cent fat, 4 per cent casein, and 4 per cent yeast) developed extremely fatty livers and among these animals there was the highest mortality after inhalation exposure. Reducing the fat content to 10 per cent afforded little protection against the toxic effects of the hydrocarbon. Increasing the casein to 47 per cent (the dietary fat remaining at 40 per cent), on the other hand, reduced the mortality almost to the level of the control rats. Choline at a level of 0.7 per cent of the diet was only partially effective but rats receiving a combination of choline and methionine, each at this level, withstood the effects of repeated exposures to the hydrocarbon as well as did the control animals. The average percentages of fat in the liver, determined chemically, and the average degree of fatty infiltration, determined histologically, varied directly with the mortality rates in every group (with the possible exception of the group of animals receiving methionine, where the mortality rate may have been somewhat low as compared with the fattiness of the liver). This finding is of particular interest in view of the fact that 1,2-dichloroethane does not produce hepatic necrosis as does chloroform or carbon tetrachloride.

In their later experiments, Heppel and co-workers studied the protective effect of various compounds, including methionine, on mice given a diet of Purina dog checkers supplemented with greens and exposed to inhalation of 1,2-dichloroethane. Dietary fatty livers did not develop although, following exposure, slight fatty degeneration of the liver was found in a number of these animals. The material to be tested for protective activity was given by stomach tube between thirty and sixty minutes before beginning the inhalation exposure. Effective protection, as measured by survival, was afforded by methionine at 1500 mg. per kilogram of body weight, by para-aminobenzoic acid at 2000 mg. per kilogram, by sulfanilamide at 1000 mg. per kilogram, and by aniline at 500 mg. per kilogram. The authors mention also that rats as well as mice could be protected in a similar way by the administration of methionine but that choline, administered immediately before exposure, had no effect. It is clear that in these cases the protection given by the methionine did not depend on its lipotropic activity.

The mechanism involved in the protection of experimental animals against the toxic effects of halogenated hydrocarbons by methionine, sulfanilamide, aniline, or para-aminobenzoic acid is not well understood. It appears that here is a fruitful field for further study. The demonstration that methionine may act as a protective agent in two distinct and apparently unrelated ways will serve to clarify some otherwise confusing issues.

NUTRITIONAL MACROCYTIC ANEMIA OF PREGNANCY

Until recently, it was generally believed that the basic nutritional deficiency in Addisonian pernicious anemia and in certain macrocytic anemias usually associated with pregnancy were essentially the same.

Theoretically, anemia of the Addisonian type might occur in pregnancy as the result of (1) a temporary depression of secretion of the specific intrinsic factor of the gastric mucosa; (2) a deficient intake of the extrinsic factor; or (3) a diversion to the fetus of the antianemic factor itself. The treatment of the macrocytic anemia of pregnancy has usually been considered identical with that of Addisonian pernicious anemia except that in the macrocytic anemia of pregnancy the treatment can be discontinued after delivery, when the demands of the fetus no longer exist.

However, during the last five years a number of investigators have made observations which strongly indicate that the macrocytic anemia of pregnancy is due to a deficiency of factors other than those needed in Addisonian pernicious anemia (Napier *et al.*, *Indian Med. Gaz.* **73,** *385 (1938);* Wills and Evans, *Lancet* **II,** *416 (1938);* Davidson, Davis, and Innes, *Brit. Med. J.* **2,** *31 (1942);* Miller and Studdert, *Lancet* **II,** *332 (1942);* Fullerton, *Brit. Med. J.* **1,** *158 (1943);* and Nielsen, *Acta med. Scandinav.* **108,** *421 (1941)*). It was found that the macrocytic anemia of pregnancy was cured by crude liver preparations but that no blood regeneration took place if purified liver preparations, highly active against the Addisonian pernicious anemia, were injected. Fullerton, for instance, found that administration of such purified liver preparations by vein to patients with macrocytic anemia of pregnancy gives rise to an increase in reticulocytes but does not affect the red blood cell count or the hemoglobin concentration. If, on the other hand, the patients were fed whole liver, the reticulocytosis was followed by a marked increase in hemoglobin and red cells. Watson and Castle (*Proc. Soc. Exp. Biol. Med.* **58,** *84 (1945)*) have described 3 cases of nutritional macrocytic anemia during pregnancy. All 3 patients gave histories of dietary inadequacy. They did not show any neural manifestations. The hematologic data demonstrated a marked reticulocyte response to various orally administered liver extracts. However, these liver preparations failed to give any reticulocyte response when administered parenterally. In Addisonian pernicious anemia, liver extracts show much greater activity when injected than when administered orally. The authors therefore suggest the possibility that the digestive organs form a new hematopoietic substance from the liver extract active against the macrocytic anemia of pregnancy. Inasmuch as the same extracts which fail to give response in the macrocytic anemia of pregnancy when administered parenterally are able to cure the Addisonian pernicious anemia when administered by the same route, there must be a basic difference between the two types of anemia.

Watson and Castle emphasize "the desirability of a therapeutic trial of orally administered liver extract in macrocytic anemias, particularly those of pregnancy, when they are refractory to parenterally administered liver extracts."

ANALYSIS OF PYRIDOXINE AND ITS DERIVATIVES

In 1942 Snell, Guirard, and Williams (*J. Biol. Chem.* **143,** *519 (1942)*) noted that the vitamin B_6 content of natural materials appeared very different when measured by the yeast assay as compared with Streptococcus lactis assay. The term "pseudopyridoxine" was employed for the material which appeared to be more active

than pyridoxine. Later, pyridoxal and pyridoxamine were recognized as derivatives which are much more active than pyridoxine itself in the S. lactis assay (Snell, *Ibid.* **154,** *313 (1944);* Harris, Heyl, and Folkers, *Ibid.* **154,** *315 (1944)*). These new derivatives showed the same activity as pyridoxine in the yeast assay, but over five thousand times the activity of pyridoxine in the S. lactis assay.

Snell and Rannefeld (*J. Biol. Chem.* **157,** *475 (1945)*) have now reported their detailed study of the vitamin activity of pyridoxal and pyridoxamine for a series of seventeen different organisms including various bacteria, yeasts, molds, and white rats. In general these compounds were either equal in activity toward these organisms or greater than pyridoxine, supporting the original contention that they are more closely related chemically to the active catalytic form of pyridoxine. It seemed questionable whether pyridoxine itself can be utilized at all by lactic acid bacteria, since growth of these ceases before maximum rates are reached even in the presence of excess pyridoxine. In two species of yeast, pyridoxamine showed somewhat less activity than pyridoxine, while pyridoxal possessed activity equal to pyridoxine. It was evident however that the apparent activity of these compounds differed due to such factors as the rate of their absorption into the organism, since their relative activity changed as the incubation time was increased.

The relative activity of these compounds toward microorganisms is complicated by their instability and the changes which may occur during autoclaving. Under conditions favoring oxidation, pyridoxine appears to be partially oxidized to an inactive product, and in dilute solution all three compounds are sensitive to light inactivation (Cunningham and Snell, *J. Biol. Chem.* **158,** *491 (1945)*). Autoclaving of these compounds with amino acids produced changes in the activity of the medium for several organisms. In choosing test organisms to assay natural materials for vitamin B_6 activity, it is therefore evident that only those which respond equally to pyridoxine, pyridoxal, and pyridoxamine are suitable. This requirement is fulfilled by the white rat, Saccharomyces carlsbergensis, and Neurospora sitophila.

Evidence had been lacking that the more active pyridoxal and pyridoxamine were actually the compounds in natural products accounting for "pseudopyridoxine" activity. The previous survey of the activity of pyridoxine, pyridoxal, and pyridoxamine toward different organisms has permitted Snell (*J. Biol. Chem.* **157,** *491 (1945)*) to measure the activity of each in a given natural material, and to compare the chemical characteristics of these fractions with the pure material. The three organisms chosen for differential analysis were S. carlsbergensis, S. faecalis, and L. casei. L. casei was sensitive nearly exclusively to pyridoxal, S. faecalis was stimulated three times as much by pyridoxamine as by pyridoxal (and negligibly by pyridoxine), and the yeast reacted nearly equally to all three compounds. Assaying a given material with all three organisms and applying the principle of simultaneous equations should yield values for the pyridoxal, pyridoxamine, and pyridoxine fraction of the material. In this way yeast extract, liver extract, rice bran concentrate, and powdered dehydrated grass juice were analyzed. With yeast, liver, and grass the pyridoxamine fraction predominates, with pyridoxal constituting only one tenth to one quarter of the total vitamin B_6 activity. With rice bran, pyridoxine appears to be in highest concentration.

Similar chemical treatments were performed on the pure pyridoxine derivatives and on the natural materials, followed by assay with the three organisms. Treatment with nitrous acid did not affect pyridoxal or the pyridoxal fraction of natural materials, while treatment with sodium cyanide

and ammonium chloride or with alkali and acetone inactivated both pyridoxal and the pyridoxal fraction. Autoclaving pyridoxal with hydrolyzed casein appears to form pyridoxamine, and with natural materials the pyridoxamine fraction also appears to increase with this treatment. Parallel results were obtained with pyridoxamine and the pyridoxamine fraction on autoclaving with alpha-ketoglutaric acid which appears to convert the amine derivative to the aldehyde form. All of the results support the contention that pyridoxal and pyridoxamine occur naturally, and that they may in some products constitute a large fraction of the total vitamin B_6 activity. Snell points out that these compounds do not necessarily occur as such, but possibly as component parts of more complex structures.

Johnson, Hamilton, and Mitchell (*J. Biol. Chem.* **158,** *619* (*1945*)) have analyzed for the excretion of pyridoxine, "pseudopyridoxine," and 4-pyridoxic acid in the urine and sweat. The latter compound had been isolated and characterized by Huff and Perlzweig (*Ibid.* **155,** *345* (*1944*)) and a fluorometric method provided. Pyridoxine was determined by a S. carlsbergensis assay, and "pseudopyridoxine" by a S. faecalis assay, using pyridoxal as a standard. There may be some question as to the specificity of these methods since the results of Snell and co-workers just reviewed indicate that yeast assay measures both pyridoxine and the "pseudopyridoxine" compounds pyridoxal and pyridoxamine, and that S. faecalis measures pyridoxamine and pyridoxal, principally the former. However, it was found that the principal vitamin B_6 excretion product both in urine and sweat was 4-pyridoxic acid, pseudopyridoxine excretion being nearly negligible. Approximately 85 per cent of the total pyridoxine and metabolites excreted was in the form of 4-pyridoxic acid, and this proportion held in the over 50 per cent of pyridoxine and its derivatives excreted after pyridoxine administration.

THE EFFECT ON GROWTH OF A SUBSTANCE IN PANCREAS

In the course of an investigation of the nutritive quality of various proteins, White and Sayers (*Proc. Soc. Exp. Biol. Med.* **51,** *270* (*1942*)) tested the value of two pancreatic protein preparations (Kazal *et al.*, *Ibid.* **51,** *268* (*1942*)) for growth of the young rat. One of these crude preparations (P-1), an acetone extracted acid-alcohol precipitate of pancreas, was fed to weanling male albino rats at the level of 22 per cent of the diet. The rats receiving this protein gained weight at a more rapid rate than did controls which were fed similar quantities of proteins from various other sources. Thus the animals on P-1 exhibited an average daily weight gain of 5.9 g. during the first twenty-eight days of feeding, in contrast to an average gain of 4.4 g. for the rats whose diets contained 21.4 per cent of a commercial casein. This difference in rate of gain was not due to a more efficient utilization of the food by the more rapidly growing group, but was accompanied by an increased food consumption. The ratio of body weight to body length was similar for all groups of rats. Bosshardt, Ciereszko, Buffington, and Arnow (*Arch. Biochem.* **7,** *1* (*1945*)) have extended these findings to mice. When casein or P-1 was fed at a level of 20 per cent of the diet and the water soluble vitamins were supplied by a mixture of several pure B-vitamins and choline plus a liver extract, the two groups of mice grew at the same rate. However, if liver extract was omitted a definite difference in growth rate was observed between the animals fed P-1 and casein, even when thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, p-aminobenzoic acid, inositol, choline, biotin, and "folic acid" were given along with the

known fat soluble factors. These results suggest, therefore, that liver extract and P-1 contain a similar or identical factor which is capable of increasing the growth rate of mice fed on casein as a source of protein.

Attempts were made to concentrate this active material and it was found to be soluble in dilute acid and in an acid-acetone mixture. The authors report a personal communication from Dr. Abraham White indicating that he has separated from P-1 a dialysable material which, when added to a casein diet, increased the growth of rats. These observations support the hypothesis that the initially observed accelerated growth rate resulting from the feeding of pancreatic protein is due to some accessory factor carried by the preparation rather than due to a peculiarity of the protein itself. Furthermore, Bosshardt and co-workers have found that the growth acceleration may be observed even in mice fed on a 35 per cent casein diet, hence it seems unlikely that this is solely an effect of protein.

A point worthy of consideration is whether the greater growth reported by these two groups of workers is truly accelerated growth which results in earlier maturity (and possibly shorter life?) of the animals, or whether it is maximum growth rate which is but realizing to the fullest the growth potentiality of the animals. In other words, is the material which produces this effect to be classified as a toxic substance or as a beneficial factor? This question cannot be answered until one knows more concerning the chemical nature and the physiologic effects of this active material from pancreas and liver.

It should be recalled, however, that similar or even more rapid growth rates have been observed for rats maintained on rations which contained neither liver nor pancreas. Anderson and Smith (*Am. J. Physiol.* **100,** *511 (1932)*), employing the Wistar Institute strain of rats fed ad libitum on a diet containing cod liver oil, linseed oil meal, corn meal, malted barley, red dog flour, dried skim milk, oat flour, soluble blood meal, sodium chloride, ground limestone, steamed bone meal, casein, whole milk powder, wheat embryo and lard, and supplemented with lettuce and yeast, have recorded average daily weight gains of 6.1 ± 0.65 g. during growth from 60 to 200 g., and of 6.0 ± 0.90 g. during growth from 60 to 300 g. Individual growth rates over a four week period were observed as high as 9.5 g. per day. It is significant that these data are on the same colony which was housed in the same room as those animals used in earlier experiments reported by Mendel and Cannon (*J. Biol. Chem.* **75,** *779 (1927)*) in which the average daily gain was 5.3 g. The only difference was in the diet. The fact that such growth rates have resulted from high protein, high vitamin, high mineral diets may indicate that practically all of the commonly used laboratory diets fail to meet the requirements for maximum growth of the rat. If so, this may account for the better performance records which have been reported after the supplementation of animals on less abundant stock diets. What is more, these results emphasize the probable inadequacy for maximal growth of almost all of the purified diets routinely employed in animal experimentation, especially those diets containing but 18 per cent casein (Zucker and Zucker, *Proc. Soc. Exp. Biol. Med.* **55,** *136 (1944)*). It is of interest that the casein-containing diets used by White and Sayers and by Bosshardt *et al.* to compare with their diets containing P-1 were slightly lower in protein content than that found by Zucker and Zucker to permit maximal growth of rats. As mentioned above, however, the latter workers produced evidence that this does not seem to account for the effect of P-1 on growth rate.

A word of caution in the interpretation of such data seems called for. "Optimal" nutritional requirements have often been loosely discussed in terms of the needs for attaining the most rapid growth rate. It is desirable to guard against such unqualified

use of this term because it is by no means proved that maximal growth rate is associated with maximal longevity or maximal resistance to disease. Hence, if the term "optimal" is used in such discussion it must always be defined as optimal for obtaining a given result, which result may or may not be desirable.

A DIETARY FACTOR FOR TREATMENT OF HYPERTENSION

It has been demonstrated that the administration of renal extracts or of certain oxidized marine oils brings about a lowering of the blood pressure in animals with induced hypertension and in patients with so-called essential hypertension. The basis of this effect is not entirely clear. It has been suggested on plausible grounds that hypertension may be in some instances a deficiency disease, resulting from renal injury, and that the kidney extracts and marine oils may act by supplying the missing factor or factors.

Harrison, Grollman, and Williams (*Am. J. Physiol.* **128,** *716 (1940)*) prepared extracts from kidneys which, when given either orally or parenterally to rats rendered hypertensive by surgery or by renin, caused a lowering of the blood pressure. No significant effect on the blood pressure of normal rats was observed. A few patients were treated with renal extracts and in most instances a decline in the blood pressure followed (Grollman, Williams, and Harrison, *J. Am. Med. Assn.* **115,** *1169 (1940)*). Similar results were obtained independently in other laboratories.

The effectiveness of marine oil concentrates in the treatment of essential hypertension in man (Pena and Villaverde, *Rev. Cubana Cardiol.* **2,** *322 (1940)*) and of surgically induced hypertension in dogs (Wakerlin, Moss, and Smith, *Science* **96,** *161 (1942)*) was at first attributed to vitamin A. Grollman and Harrison (*Proc. Soc. Exp. Biol. Med.* **52,** *162 (1943); Nutrition Reviews* **2,** *240 (1944)*), demonstrated however, that the antipressor activity of such concentrates is not related to their vitamin A content. Employing as test animals rats rendered hypertensive by surgical fixation of the kidneys, they tested carotene and various preparations and concentrates of fish oils, before and after destruction of the associated vitamin A. Carotene had no effect. Various fish liver and body oils were effective but the activities of different preparations were not proportional to their vitamin A content. Oxidation by peroxide or chromic acid, while destroying the vitamin, resulted in some cases in actual increases in antipressor activity. Where an increase of this type occurred, there was no relationship between its magnitude and the original vitamin A content of the preparation. On the basis of these results, the authors concluded that vitamin A was neither the antipressor substance nor its precursor. Moss and Wakerlin (*Federation Proceedings* **3,** *34 (1944)*) have reported that further lots of the vitamin A concentrate which they originally employed proved to have no antipressor activity and they agree that the active principle is not vitamin A.

Considerable additional information is now available concerning the distribution of the antipressor substance and its precursor and concerning methods for the concentration of the active material. Grollman (*J. Pharmacol. Exp. Therap.* **84,** *128 (1945)*) has shown that most samples of cod liver oil as they are obtained commercially are inactive. Other marine oils showed varying activity. Oxidation increased the activity of some samples, for others saponification followed by oxidation was necessary, and for many others neither treatment caused any increase. Of the vegetable oils tested, only tung oil showed unquestionable activity. The most effective concentration procedure developed was adsorption on amberlite, removal of adherent oil with skellysolve, and

elution with potassium hydroxide. In this manner the active principle was obtained in aqueous solution. It was shown to be dialysable. Distillation was not uniform in its effects and the expected concentration was not realized. The author concluded that the active substance is probably a derivative obtained by oxidation of an unsaturated fatty acid or possibly of some other esterifiable substance. He suggested that it might be identical or of related chemical structure to the active substance in renal extracts.

Grollman also discussed the results of the use of an active oxidized oil on a limited number of hypertensive patients. One case in particular, of hypertension secondary to chronic glomerulonephritis, gave convincing results. On two separate occasions treatment reduced the systolic blood pressure slowly over approximately three month periods, from 200 mm. of mercury or higher to below 150. Substitution of a placebo for the active oil permitted the pressure to return very slowly to the 200 mm. of mercury level.

The results so far obtained must be classed as promising. As emphasized by Grollman, however, sufficient material is not yet available for adequate clinical trial and the use of both oils and renal extracts must still be regarded as purely experimental.

AN EFFECT OF DIFFERENT CARBOHYDRATES AND FATS ON B-VITAMIN REQUIREMENTS OF THE RAT

Several aspects of the controversy as to the relative nutritional value of corn oil and butterfat have been discussed previously (*Nutrition Reviews* **1,** *122, 358 (1943);* **2,** *165, 267, 351 (1944)*). To recapitulate briefly, the evidence indicates that when differences in growth have been observed between animals receiving corn oil and those fed butterfat it has been under rather stringent experimental conditions (Schantz, Elvehjem, and Hart, *J. Dairy Sci.* **23,** *181 (1940)*; Freeman and Ivy, *Ibid.* **25,** *877 (1942)*; Geyer, Boutwell, Elvehjem, and Hart, *Science* **98,** *499 (1943)*). When various carbohydrates were used in the diet, it was only in the presence of a high percentage of lactose that there was observed a greater rate of growth by rats fed on butterfat than in animals fed on corn oil. Boutwell, Geyer, Elvehjem, and Hart (*J. Dairy Sci.* **26,** *429 (1943)*) suggested that this difference might be due to an alteration of the intestinal flora brought about by the feeding of large amounts of lactose.

A further investigation of this suggestion has now been reported by these latter workers (*Arch. Biochem.* **7,** *143 (1945)*). Twenty-day old male albino rats of the Sprague-Dawley strain were fed ad libitum for six weeks and the weight change noted. The rations consisted of carbohydrate 48, ethanol and ether extracted casein 20, salt mixture 4, and fat (butterfat or corn oil) 28. Vitamin supplements of thiamine, riboflavin, pyridoxine, pantothenic acid, and choline were fed at three levels: medium, high, and high plus liver extract. The medium supplements were at a level frequently employed satisfactorily in similar purified diets. The high levels were some two and a half times the medium and contained in addition niacin, inositol, and p-aminobenzoic acid. Liver extract, where fed, was as a whole powder at the level of 1 g. per 100 g. of diet. The usual fat soluble vitamins were supplied. Various carbohydrates employed were: galactose, glucose, corn starch, dextrin, Dexti-maltose, sucrose, fructose, alpha-lactose, beta-lactose, and two mixtures of equal parts of glucose and galactose or glucose and fructose. The effect of diets containing each carbohydrate in combination with each of the fats at all three vitamin levels was tested.

Three to 60 rats per group were used. The final growth data for the six week period were tabulated.

An examination of the mean weight increases for the period reveals that the lactose and the galactose fed rats gained less at all levels of vitamin feeding than did rats receiving other carbohydrates, although increased gains occurred as the intake of vitamins was increased. A definitely greater weight gain occurred in the butterfat group compared with the corn oil groups at all three vitamin levels when alpha-lactose comprised 48 per cent of the diet. This was the only carbohydrate tested in which such a difference was observed on the high vitamin plus liver extract supplement. With the omission of the liver extract but maintaining a high vitamin intake, greater gains on the butterfat diet in comparison with the corn oil regimen were also manifested by those animals getting sucrose or Dexti-maltose. Finally, at the medium (lowest) level of intake of vitamins the rats which were fed butter were heavier than the corn oil group on all carbohydrates tested except glucose and galactose-glucose mixture. In addition to the weight differences, the general appearance of rats fed the corn oil and lactose was poorer than those fed butterfat and lactose irrespective of the vitamin levels. This was also true at the medium vitamin level for other carbohydrates. The observed signs were "bloody" nose and head, poor fur, and extensive symmetrical abdominal alopecia. These findings indicate that the presence of butterfat in certain purified diets may, in some manner, lower the exogenous requirements of the rat for vitamins of the B-complex necessary for maximum growth below that of a similar diet containing corn oil. It may be, therefore, that differences in the B-vitamin supplements might account for some of the discordant results which have previously appeared in work on this problem. An outstanding example of such a phenomenon a few years ago was the observed difference in growth response of rats maintained on a methionine free diet and supplemented with homocystine (Rose and Rice, *J. Biol. Chem.* **130,** *305 (1939)*; du Vigneaud, Dyer, and Kies, *Ibid.* **130,** *325 (1939)*). The observed discrepancy proved to be due to the presence of choline (a source of labile methyl) in the vitamin source used by one experimenter and the absence of it in the vitamin supplement used by the other (du Vigneaud, Chandler, Moyer, and Keppel, *Ibid.* **131,** *57 (1939)*).

The Wisconsin investigators attribute the observed differences to the more favorable influence of butterfat on intestinal synthesis of some undefined B-vitamin or vitamins. They report *in vitro* experiments with L. casei and S. lactis in the presence of the two fats to support the reasonableness of such an explanation. It does not appear, however, that differences in metabolic requirements were completely ruled out by these experiments.

The workers ask the question, "Would man subsisting on a diet low in the water-soluble vitamins be further endangered by the consumption of vegetable oils or their products as compared with animal fats, especially butterfat?" The raising of this interesting question is not to be taken as an implication that the answer is in the affirmative. In fact, in practice it is difficult to conceive of a human dietary which might obtain some 48 per cent of its calories from vegetable fats, be devoid of animal fat and, at the same time, contain a large amount of such pure carbohydrates as employed in these experiments. The value of this report lies in its seeming clarification of a discrepancy in experimental findings in a controversial field, not in any demonstration of a superiority of one fat over another under conditions met with in practical nutritional work with man.

THE VIRUCIDAL ACTION OF ASCORBIC ACID

The virucidal action, i.e. the inhibition of virus growth, of ascorbic acid has been reported by a large number of investigators. Jungeblut (*J. Exp. Med.* **62,** *517 (1935)*) observed the virucidal effect of ascorbic acid on poliomyelitis virus, and Knight and Stanley (*Ibid.* **79,** *291 (1944)*) more recently described the same effect on influenza A virus. It is important to know something of the chemical mechanism involved in this effect. Furthermore, it is important to know whether this action is related to the specific biologic characteristics of ascorbic acid, i.e. its function as a vitamin, or whether it results from some other chemical characteristic of the substance. Klein (*Science* **101,** *587 (1945)*) has recently reported further investigation on the virucidal effect of ascorbic acid on influenza A virus.

It has been known for some time that hydrogen peroxide is a product resulting from the oxidation of ascorbic acid in air (Lyman, Schultze, and King, *J. Biol. Chem.* **118,** *757 (1937)*). It now appears from the following observations that hydrogen peroxide is the active virucidal agent: (1) This virucidal effect of ascorbic acid is abolished by the presence of small amounts of catalase, the enzyme which specifically destroys hydrogen peroxide; (2) concentrations of hydrogen peroxide approximating those resulting from the spontaneous oxidation of ascorbic acid in aqueous solution were found to inactivate influenza A virus.

The PR8 strain of influenza A virus used in the study was grown in the allantoic fluid of chick embryos. Tests for viral activity were performed by nasal instillation of 0.05 ml. saline dilutions into mice, and recording the deaths from lobar pneumonia over a ten day period.

Administration of either 10 or 100 MLD (Minimal Lethal Dose) of untreated virus resulted invariably in death from typical pneumonia on the sixth, seventh, or eighth day. If, however, these doses were incubated for ten minutes at room temperature with 1.0 ml. of a 0.05 M. solution of ascorbic acid before instillation, the effect of 10 MLD of virus was nil, and there was a strong inhibition of the effect of the 100 MLD dose of virus. When the virus was incubated with ascorbic acid plus catalase the same number of animals succumbed, as was the case with the untreated virus. Catalase plus ascorbic acid together or separately had no effect on the mice.

The author found that if hydrogen peroxide was substituted for ascorbic acid the virus was likewise inactivated. The minimal concentration of hydrogen peroxide causing complete inactivation of 10 MLD of influenza A virus was 0.1 per cent, which approximated the amount of peroxide formed by oxidation of ascorbic acid during the ten minute incubation period. Unfortunately, it is not stated how long a time the virus was incubated with hydrogen peroxide, but it was presumably ten minutes as was the case with ascorbic acid. The inactivation by hydrogen peroxide was nullified, as would be expected, by the presence of catalase. It seems likely, therefore, that the ascorbic acid effect is attributed at least in large part to the formation of hydrogen peroxide.

McCarty (*J. Exp. Med.* **81,** *501 (1945)*) has described an analogous inhibiting action of ascorbic acid. While studying the nucleic acid which transforms type III pneumococcus into type II (Avery, MacLeod, and McCarty, *Ibid.* **79,** *137 (1944)*) it was found that the transforming factor was slowly inactivated by small amounts of ascorbic acid in the presence of oxygen and that this inactivation was considerably augmented by addition of copper sulfate which had no effect by itself. The presence of sulfur containing amino acids or peptides such as cysteine or glutathione prevented the inactivation of the factor by ascorbic acid. Moreover, if the factor was first inactivated

by ascorbic acid and copper, it was possible to reactivate it by the addition of glutathione. Inasmuch as minute amounts of catalase prevented the inactivation by ascorbic acid, it was naturally assumed that the effect was due to oxidation by hydrogen peroxide. McCarty found, however, that the amounts of hydrogen peroxide required for the inhibition of the transforming factor greatly exceeded the amounts which could be formed from the concentrations of ascorbic acid which inactivated the factor. He therefore suggests as another alternative that an intermediate oxidation compound of ascorbic acid causes the inactivation of the transforming factor. It was also found that the inactivation of the transforming factor could be accomplished by glucoascorbic acid and isoascorbic acid, and a number of other dienol compounds. It is clear, therefore, that this effect of ascorbic acid has nothing to do with its functions as a vitamin.

The inactivation of high molecular substances by oxidation, particularly by hydrogen peroxide, is a phenomenon which is frequently encountered, i.e. many enzymes are inactivated by traces of hydrogen peroxide and some of these can be reactivated by addition of cysteine or glutathione.

It is also of interest to note that certain oxidizing enzymes like glucose oxidase (so-called penicillin B) form hydrogen peroxide and that the latter inhibits greatly the growth of bacteria (Michaelis *et al.*, *Nature* **150,** *634 (1942)*; Kocholaty, *Arch. Biochem.* **2,** *73 (1943)*; Roberts *et al.*, *J. Biol. Chem.* **147,** *47 (1943)*).

The prospects of using the virucidal or bactericidal effect of hydrogen peroxide producers therapeutically are probably scant. The body tissues contain large amounts of catalase and any hydrogen peroxide formed would probably be decomposed by catalase before it had an opportunity to react with any virus or bacteria.

GENETIC FACTORS IN SYNTHESIS OF B-VITAMINS BY LOWER PLANT FORMS

One means of improving the nutritive quality of the diet of the general population is through the production of natural plant foods richer in vitamins and other essential dietary factors. It has been shown that both environmental conditions and genetic constitution are important factors in the vitamin content of plants and their products. Considerable progress in this direction has been made, particularly with respect to the carotene and ascorbic acid content of fruit and vegetables (*Nutrition Reviews* **3,** *216 (1945)*). Exploration of similar possibilities for the vitamin B factors is still in the initial phases.

Yeasts have served as particularly promising experimental plants for genetic studies on B-vitamins because of the rapidity with which results may be obtained on these fast growing organisms. Survey studies of yeast strains have revealed great variation with respect to their ability to grow in the absence of certain members of the B-complex (Burkholder, McVeigh, and Moyer, *J. Bact.* **48,** *385 (1944)*). Tests of one hundred and fifty strains of yeast on synthetic media containing all the vitamins and then on the basal medium lacking in one B-vitamin at a time, formed the basis for rating the strains as to whether or not they required the lacking vitamin. Strains able to grow well in a nutrient lacking a vitamin are assumed to synthesize that vitamin. One strain isolated from sour milk proved to be particularly efficient in the production of riboflavin. Burkholder (*Yale Scientific Monthly* **18,** *6 (no. 1) (1943)*) points out the economic potentialities in the development of giant and hybrid yeasts which possess valuable and desirable characteristics. The giant yeasts resulting from multiplying the chromosome number in cells (polyploid) are sometimes

very efficient in making protein from molasses and ammonia.

Hybrid yeasts possessing enhanced capacities in synthesizing the B-vitamins have resulted from the experiments of Lindegren and Lindegren (*Science* **102,** *33* (*1945*)). Two instances are reported where the crossing of species of yeasts has resulted in the production of hybrid strains able to synthesize vitamins which one of the parent strains could not synthesize. A haplophase culture derived from a strain of Saccharomyces cerivisiae which failed to synthesize pantothenic acid and biotin but synthesized pyridoxine well, was mated with a haplophase culture of Streptococcus carlsbergensis which synthesized the former factors well but lacked the ability to synthesize the latter. The resulting hybrid synthesized all 3 vitamins in nearly as large quantities as the parent haploid cultures. Dissection of the ascospores of the hybrid indicated it to be heterozygous for the production of all 3 factors. The diploid culture of S. cerivisiae from which the haploid was derived was heterozygous for the gene pair controlling pantothenic acid synthesis.

Similarly, a haploid culture of S. globosus which was capable of synthesis of pantothenic acid and incapable of synthesis of thiamine, was mated with a haploid from a homozygous pantothenic acid negative culture of S. cerivisiae. One resulting hybrid was backcrossed to the original S. cerivisiae. Thus, a diploid was produced capable of synthesizing both pantothenic acid and thiamine efficiently. It is interesting to note that neither parent could synthesize both vitamins. Hence the hybrid obviously obtained its ability for synthesis of pantothenic acid from S. globosus and of thiamine from S. cerivisiae. It inherited from both parent strains poor ability in biotin synthesis.

The existence of significant hereditary differences in these lower forms of plants with respect to their capacity to synthesize B-vitamins constitutes a basis for similar genetic factors in higher plants. It has been shown that strains of spring wheat grown in varying soils of the Canadian prairies are consistently different in their thiamine content (Newman, *J. Am. Soc. Agron.* **34,** *109* (*1942*)). Likewise winter wheat varieties grown on the plains of the United States exhibit differences in thiamine content. Wide variations also exist in the niacin content of corn to which reference has been made (*Nutrition Reviews* **3,** *26* (*1945*)). It seems feasible through study of the appropriate genetic and environmental factors to develop varieties of these basic food plants and others which contain greatly enhanced levels of the B-vitamins.

BIOCHEMICAL METHODS IN NUTRITIONAL SURVEYS

Precision in estimating the part played by nutrition in health has been limited in part by lack of methods for accurate biochemical analyses carried out on large population samples. Even with the inherent difficulties in correlating and evaluating the measurable components of health which are influenced or governed by nutrition, lack of simple but reliable chemical procedures for processing larger numbers of subjects has been one of the chief limiting factors in surveys of nutritional status. Thus ample data have not been accumulated to establish a satisfactory bench mark of average vitamin contents and excretion rates for representative portions of the entire population. Complexity of existing methods and excessive requirements for apparatus, reagents, and technically skilled investigators have restricted most of the observations to those on patients or healthy persons in or accessible to hospitals and laboratories. Only when the laboratory goes into the field does it overcome these handicaps.

Bessey and Lowry (*Am. J. Pub. Health* **35**, *941 (1945)*) have given a general report which discusses the type of information they have been able to obtain by developing microtechnics which permit them to determine vitamin A, carotene, ascorbic acid, riboflavin, serum protein, hemoglobin, and phosphatase on 0.1 ml. of blood drawn from the finger tip. The methods have been thoroughly standardized, are accurate and reproducible. Details of the methods and proof of their reliability are being prepared for publication. A brief sketch of the tools, time, and procedures indicates that 25 specimens per hour can be obtained by two people, 50 cu. mm. of blood being collected into a capillary tube containing heparin and 50 cu. mm. collected without heparin and allowed to clot. Ascorbic acid is determined on 10 cu. mm. of serum by the dinitrophenyl hydrazine reaction. Three cu. mm. of serum are used to measure phosphatase by a newly developed method. Two to 5 cu. mm. of serum are used to determine protein by use of the Linderstrøm-Lang gradient tube. Vitamin A and carotene are measured on 35 cu. mm. of plasma after saponification and extraction. Riboflavin is determined on 15 cu. mm. of red cells by rate of acid production by Lactobacillus casei, and hemoglobin is measured on 10 cu. mm. of whole blood after dilution by light absorption in a photoelectric colorimeter. Some of the apparatus cannot be carried into the field, but specimens can be frozen and shipped to the laboratory for analysis. The field kit for an extensive survey weighs only 100 pounds. Two advantages of the micromethod are the possibility of studies on small animals and the frequent repetition of studies on human subjects without significant removal of appreciable quantities of blood.

A presentation of two charts giving distribution curves on ascorbic acid and hemoglobin values for three groups of New York schoolchildren, prosperous, intermediate, and poor, indicates the value of such data in separating groups. Further study of such material, and multiple correlations on individual subjects' data, particularly as observed over a period of time, hold promise of valuable additions to the knowledge of biochemical aspects of nutrition.

Another approach to the methodology of the mass survey in nutrition has been expounded by Johnson and associates ("Laboratory Manual of Field Methods for Biochemical Assessment of Metabolic and Nutritional Condition," *Harvard Fatigue Laboratory, Boston (1945)*). In essence it represents the accumulated experience of these workers in various ration trials conducted by the United States and Canadian Armed Forces from 1942 through 1944. The chief difference in procedure from that of Bessey and Lowry is that the entire laboratory, apparatus, reagents, and technical assistants, is transported into the field and carries out its biochemical determinations in any convenient location where blood and urine samples can be collected. Its logistical requirements are those of an independent mobile unit which can either set up in a semipermanent camp or follow a tactical unit on maneuvers. Equipment is packed in small, rugged boxes of a size readily handled by one man. Specifications and details of apparatus, chemicals, and procedures are outlined, and numerous illustrations enhance the value of the manual.

A well trained team can collect samples on 70 men in an hour; and a team of no more than eight field workers has been able to test 600 men in six days, running determinations on hemoglobin, serum protein, serum and urinary chloride, and fasting urinary ascorbic acid, thiamine, riboflavin, and F_2 factor with load or tolerance tests for the vitamins in half the subjects. Such tests have been repeated four times within two months.

Explicit details of methods and procedures are outlined for each stage of the various determinations. It is obvious that for study of such large numbers of persons the assignment of specific duties to each member of the team, precise scheduling of bleeding times

and urine collection, handling test subjects, labeling, collecting, storing, and processing of specimens requires meticulous planning and much practice before satisfactory results can be expected.

One of the most valuable features of the Harvard Fatigue Laboratory Manual is the series of tables, nomograms, and other data which facilitate calculations and give a basis for comparison and evaluation. In addition to the study of vitamins and other constituents of blood and urine noted above, methods for minerals, glucose, nonprotein nitrogen, blood gases, and respiratory gases are detailed. Thus with the information presented it is possible to conduct a comprehensive laboratory study of nutrition and respiratory metabolism in the field. This has been invaluable for the Army where ration trials had to be conducted with regard for simulated combat conditions and all testing done in bivouacs or on maneuvers.

Each of the two types of survey technics is best adopted to a particular set of circumstances. The methods of Bessey and Lowry may be used in studies of civilian populations where fixed hospitals and laboratories are available. The technics of Johnson and associates from the Harvard Fatigue Laboratory have been used successfully in field tests and maneuvers in the continental United States and have recently been employed successfully in conduct of one phase of an extensive survey of nutrition in garrison and combat troops in the Pacific theater of war.

THE FEEDING OF SYNTHETIC THYROPROTEIN TO DAIRY COWS

In 1942 Reineke and Turner (*J. Dairy Sci.* **25,** *393 (1942)*) published the results of experiments which showed that iodinated milk proteins were capable of increasing milk production when fed for short periods to lactating cows and goats. A similar stimulation of milk production with desiccated thyroid or thyroxine had previously been demonstrated by Graham (*J. Nutrition* **7,** *407 (1934)*, *Biochem. J.* **28,** *1368 (1934)*); Jack and Bechdel (*J. Dairy Sci.* **18,** *195 (1935)*); Folley and White (*Proc. Roy. Soc. London, Ser. B,* **120,** *346 (1936)*); Herman, Graham, and Turner (*Mo. Agr. Exp. Sta. Res. Bull.* **275** *(1938)*) and Ralston and associates (*Ibid.* **317** *(1940)*). The increase in milk production obtained by Reineke and Turner with the synthetic thyroprotein was from 0.8 to 40.8 per cent with goats, and from 6.09 to 22.6 per cent with dairy cattle, with corresponding increases in yield of butterfat.

In a subsequent report Reineke and Turner (*Mo. Agr. Exp. Sta. Res. Bull.* **355** *(1942)*) discuss experiments on the formation *in vitro*, and the assay of thyroproteins of the type used in their feeding experiments, and describe a standard procedure for their preparation. They state that with other conditions constant the thyroidal activity of iodinated casein does not reach a maximum until a pH of 6.8 to 8.0 is maintained during the iodination reaction. At the same time there is also a progressive increase in thyroidal activity until sufficient iodine has been added to combine with two atoms per molecule of tyrosine in the protein, while further iodination results in losses of activity. Elevation of the temperature to 60 to 70°C. either during iodination or during the subsequent incubation period is reported to increase the thyroidal activity of the iodinated casein, and a further increase occurs if the reaction takes place in the presence of a metal or combination of metals contained in brass. With all factors optimum, the thyroidal potency of iodinated casein is approximately 10 per cent of that of thyroxine when assayed by injection into tadpoles, and 4 per cent when tested orally on guinea pigs. As judged by tadpole assay it is approximately equal to synthetic thyroxine

when compared on an equal iodine basis, or possesses 40 per cent of the potency of thyroxine as tested with guinea pigs. Upon iodination, soybean proteins and egg albumin yield products equal in thyroidal potency to iodinated casein.

The preliminary results obtained by feeding synthetic thyroprotein and its relatively low cost indicated the possibility of the use of this material as a practical means of increasing the production of milk and butterfat. Reineke (*J. Dairy Sci.* **26,** *750 (1943)*) reported the use of thyroprotein, in amounts of approximately 1.5 to 2.5 g. per 100 pounds of body weight, with 27 cows of three different breeds, all in the declining stage of lactation. The observations covered periods up to three months, during which 23 of the 27 cows showed increases of from 6.6 to 60.8 per cent in milk production after six or more days of feeding. After one or two months of elevated production the normal decline of lactation occurred, but at a retarded rate. No undesirable effects such as hyperirritability and loss in body weight occurred. According to a later report of Reineke, Herman, Turner, and Ragsdale (*J. Animal Sci.* **3,** *439 (1944)*), a daily amount of 0.5 g. of thyroprotein per 100 pounds of body weight produced a slight but questionable rise in milk fat test but little or no effect on milk yield. An increased daily dosage of 1.0 to 1.5 g. per 100 pounds of body weight caused an increase of about 10 per cent in milk yield and an increase of 15 to 25 per cent in fat yield over a period of five months.

Further reports on the feeding of iodinated casein ("Protomone") have come from the West Virginia, Louisiana, and New Jersey Agricultural Experiment Stations. Van-Landingham, Henderson, and Weakley (*J. Dairy Sci.* **27,** *385 (1944)*), on feeding 15 g. daily of "Protomone" to cows in declining lactation, noted increases of from 5 to 20 per cent in milk production and 25 to 50 per cent in butterfat production, the latter being due both to the increase in milk production and to an increase in the fat content of the milk. Increases in the pulse rate and respiration of the cows were apparent along with some decreases in body weight. These authors reported further a decrease of about 33 per cent in the ascorbic acid content of the milk of cows fed "Protomone."

Seath, Branton, and Groth (*J. Dairy Sci.* **28,** *509 (1945)*) fed "Protomone" to cows in experiments designed to determine whether "the stimulatory effects of such feeding would adversely affect the health of milk cows in the relatively warm and humid climate of Louisiana." In general, these authors obtained results similar to those previously reported, but with one group of cows fed "Protomone," after five to six weeks of higher fat and milk yields, a decrease in production occurred below that of a control group of cows fed normally. These authors feel that it is highly questionable whether the increased production secured by feeding iodinated casein to milk cows under Louisiana conditions would be great enough or would continue over a long enough period to justify possible injury that might result from losses in body weight, increases in pulse rates, and higher body temperatures.

Reece (*J. Dairy Sci.* **27,** *545 (1944)*) has reported an experiment showing that the feeding of moderate amounts of "Protomone" will definitely increase the butterfat content of milk without causing any great increase in milk production or great loss in body weight. He points out that certain cows produce relatively large amounts of milk of low fat test, and that many cows increase in body weight at the expense of milk production. The work of Reece suggests that these characteristics of individual cows should be considered in determining the amount of "Protomone" to be fed.

In spite of the essential agreement of the results of much of this research, it would appear that the practicability of feeding thyroprotein under average conditions of dairy practice is yet to be proved. Experiments of longer duration are particularly to be desired.

ANTIMETABOLITES OF PANTOTHENIC ACID, ALPHA-TOCOPHEROL, AND PHENYLALANINE

The antivitamins or antimetabolites, substances whose chemical structures are similar to those of essential cellular catalysts, continue to draw the interest of biochemists. One of the vitamins which received early attention in this regard was pantothenic acid, for which the antivitamin "pantoyltaurine" was synthesized (*Nutrition Reviews* **1,** *234* (*1943*)). Interest in pantothenic acid analogues was no doubt increased by the finding of McIlwain and Hawking (*Lancet* **I,** *449* (*1943*)) that rats could be protected from 100,000 lethal doses of hemolytic streptococci by pantoyltaurine. Pantothenyl alcohol in which the acid group of the vitamin is replaced by the alcohol group, was tested on rats by Pfaltz (*Z. Vitaminforsch.* **13,** *236* (*1943*)). Rather than acting as an inhibitor, the alcohol proved to be as effective as pantothenic acid in preventing achromotrichia, presumably because it may be oxidized to the acid in metabolism.

Snell and Shive (*J. Biol. Chem.* **158,** *551* (*1945*)) have shown, however, that pantothenyl alcohol acts as a growth inhibitor of yeast and various bacteria, and that the inhibition is competitive in nature. In no case could the alcohol replace pantothenic acid. Leuconostoc mesenteroides P-60 proved to be most sensitive to pantothenyl alcohol, but certain lactic acid bacilli, Streptococci faecalis R and lactis 125, and Saccharomyces cerivisiae FB were also affected. Staphylococcus aureus and E. coli were unaffected. In general the effects of pantothenyl alcohol and pantoyltaurine were similar, but relative sensitivities of the organisms to these compounds differed greatly, possibly due simply to differences in permeability of the cells to the two substances. Growth inhibition by either of the substances was counteracted by pantothenic acid, and the extent of inhibition governed by the ratio of inhibitor to vitamin. Other pantothenic acid analogues were prepared and tested by Snell and Shive, but were found to be less active as growth inhibitors than pantothenyl alcohol.

The inhibitory action of still another pantothenic acid analogue has been reported by Woolley and Collyer (*J. Biol. Chem.* **159,** *263* (*1945*)). The new compound is phenyl pantothenone, a compound in which the carboxyl group of the vitamin is replaced by a phenyl ketone group. This compound proved to have a growth inhibiting effect on L. casei, L. arabinosus, a group B hemolytic Streptococcus, E. coli, Staph. aureus, Saccharomyces cerivisiae, and Endomyces vernalis. In general, the addition of pantothenic acid caused a reversal of the inhibition with those organisms which require pantothenic acid in the medium, but not those which do not require pantothenic acid for growth. These findings are similar to Woolley's previous results on the scurvy-producing effect of glucoascorbic acid on various animal species, in which it was found that ascorbic acid had a reversing effect only in guinea pigs. Of fundamental interest to the general problem of antimetabolites is the apparent fact that replacement of carboxyl groups of vitamins by methyl or phenyl ketone groups produces effective inhibitors. This contention is supported by the study with phenyl pantothenone, and the earlier studies with 3-acetyl pyridine (Woolley, *J. Biol. Chem.* **157,** *455* (*1945*)) and p-aminoacetophenone (Auhagen, *Z.f. physiol. Chem.* **274,** *48* (*1942*)).

A most interesting situation has arisen with regard to Woolley's experiments on the effect of alpha-tocopherol quinone in pregnant mice. In attempting to reproduce the results of alpha-tocopherol deficiency in pregnant mice by the administration of the analogue alpha-tocopherol quinone, Woolley (*J. Biol. Chem.* **159,** *59* (*1945*)) found that the analogue produced the expected resorption of the embryo, but that excessive vagi-

nal bleeding also occurred. Due to the latter finding, the effect of vitamin K was studied, and it was surprising to find that this vitamin prevented both the resorption and vaginal bleeding caused by alpha-tocopherol quinone. A comparison of the chemical structural formulas of alpha-tocopherol quinone with alpha-tocopherol and vitamin K showed that the new compound might be expected to possess an antagonism to both vitamins. The new compound differed somewhat from the more typical vitamin K inhibitor, 3,3′-methylene-bis (4-hydroxycoumarin), in the fact that the hemorrhages produced were confined to the reproductive system, and that no marked prolongation of prothrombin time was noted. It would appear that alpha-tocopherol quinone possesses "antimetabolite" activity toward both vitamins E and K. The selectivity of the agent toward pregnant animals would not be expected if vitamin K antagonism were solely responsible, and yet the compound did not possess the expected specificity of a vitamin E inhibitor.

The "antimetabolite" principle need not be confined to vitamins, but may also be extended to essential amino acids. Dyer (*J. Biol. Chem.* **124,** *519 (1938)*) showed that ethionine was toxic to young rats maintained on a methionine deficient diet, and Harris and Kohn (*J. Pharmacol. Exp. Therap.* **73,** *383 (1941)*) have demonstrated the reversible "antimethionine" effect of ethionine for E. coli. Du Vigneaud *et al.* (*J. Biol. Chem.* **159,** *385 (1945)*) have now reported an antagonistic effect of thienylalanine against phenylalanine in the growth of Saccharomyces cerivisiae, an antagonism which was reversed by addition of phenylalanine to the growth medium. The addition of this sulfur analogue of phenylalanine to the diet of young rats failed to replace either phenylalanine or methionine in the diet.

There seems to be no limit to the number of synthetic compounds which by virtue of their similar chemical structure might be antagonistic to vitamins and essential amino acids. Apart from the basic interest of these compounds is the possibility that some may be useful therapeutic agents. The rapid development of this general field promises eventually to reveal antimetabolites with special practical applications.

SELF SELECTION OF DIET BY RATS FOLLOWING PANCREATECTOMY

The studies of Richter and associates which have revealed a remarkably efficient self regulation of diet by rats have been followed with considerable interest. A further report (Richter, Schmidt, and Malone, *Bull. Johns Hopkins Hosp.* **76,** *192 (1945)*) offers interesting information concerning the choice of foods by pancreatectomized rats. In these experiments, rats are placed in individual cages where they may consume at will a variety of food substances. Frequent readings of the contents of the separate food containers yield accurate information regarding food consumption. Accurate records of activity and weight are also kept. In a previous study (*Endocrinology* **28,** *179 (1941)*) Richter and Schmidt had observed that pancreatectomized rats which for months while receiving a regular stock diet had manifested all the signs of diabetes, namely polydipsia, polyphagia, loss of weight, and hyperglycemia, lost these signs when permitted to select their own diets from an assortment of ten substances in separate containers. Such rats selected high amounts of fat, low quantities of carbohydrate, and variable amounts of protein in addition to large amounts of yeast. By using more purified substances it was hoped that information could be obtained concerning the appetite of pancreatectomized rats for protein and for a mixture of the crystalline vitamins which included thiamine, riboflavin, nicotinic acid, pyridoxine, calcium

pantothenate, and choline chloride. Vitamin free casein constituted the sole source of protein. The diet, unfortunately, was lacking in several components of the B-complex as well as vitamin E, vitamin D, iodine, iron, copper, and cobalt.

Eight female rats were kept on the self selection diet with the crystalline vitamin B components for at least forty days before pancreatectomy. Postoperatively they were observed on the self selection diet for at least forty days and in some instances for one hundred and thirty days, after which they were placed on a stock diet.

Before operation the rats selected large amounts of dextrose, moderate amounts of casein, and almost no cod liver oil or olive oil. For two or more weeks after operation they continued to take high amounts of dextrose and no fat. At that time, however, a sharp reversal in fat and carbohydrate appetite took place, the consumption of carbohydrate falling sharply and that of fat rising significantly. Consumption of casein increased almost at once after operation and this was associated in most instances by a twofold to threefold increase in the consumption of the crystalline vitamins. On the average the caloric intake of carbohydrate decreased from 65 to 30 per cent, the fat intake increased from 15 to 39 per cent, and the protein intake increased from 20 to 31 per cent. No significant change in mineral intake was observed. So long as the animals remained on the self selection diet they showed no symptoms of diabetes. When they were placed on the stock diet, however, and thus forced to take the various ingredients in fixed amounts, polydipsia and polyphagia appeared almost at once. Furthermore, rats which had become very inactive after pancreatectomy began to be active again on a diet which was high in protein, the B-complex, and olive oil. This occurred, however, only after the olive oil was added, suggesting that the fat was essential for activity.

The investigators' speculation as to the significance of these observations is interesting. On a stock diet when animals are unable to vary the intake of the different constituents, pancreatectomized rats eat larger amounts of the diet, possibly in order to obtain calories from the fat which they can utilize. Thus arises diabetic polyphagia. The ingested carbohydrate cannot be used by the muscles and so remains in the blood stream in large amounts. The kidneys excrete a large proportion of the excess sugar, thus producing the polyuria of diabetes. This large excretion of sugar requires excretion of water. The consequent general dehydration can be compensated for only by the intake of large amounts of water. This brings about the polydipsia of diabetes. After pancreatectomy protein is lost, as shown by the increased nitrogen output in the urine. The consequence is a loss of weight. When allowed to select the ingredients of their diets, the rats took only small amounts of the carbohydrate which they could not utilize without insulin and they increased the amount of fat and protein which they could utilize. As a result, no polyphagia developed. The components of the vitamin B-complex were taken in increased amounts, perhaps to utilize the carbohydrate and the larger amounts of protein. Since the carbohydrate intake was low, the blood sugar was probably not increased, water was not needed for the excretion of excess sugar, and polyuria and polydipsia developed only in a slight degree or not at all. The high amounts of ingested fat were transformed into energy and high levels of running activity were observed in the animals.

The preference of pancreatectomized rats for fats is of special interest since it is consistent with certain observations which were made in pre-insulin days in diabetic patients. Fat was the one unrestricted food in diabetes. It formed the bulk of the diabetic patient's diet and on such diets many did remarkably well.

BIOTIN AND REPRODUCTION AND LACTATION IN THE RAT

Kennedy and Palmer (*Arch. Biochem.* **7**, *9* (*1945*)) observed that when sows were fed on large quantities of eggs the newborn pigs were paralyzed, or survived but a few days, or were born dead. In an effort to explain this observation young female albino rats were raised on similar purified diets containing 30 per cent egg albumin or 15 per cent egg albumin and 15 per cent casein with or without added biotin. The females were mated with normal males and all litters which resulted were reduced to 4 pups and weaned at the age of 28 days. The breeding records of the animals fed on 30 per cent egg albumin indicate that in the absence of added dietary biotin or when 2 micrograms of biotin per day were added there was failure to produce a second litter because of resorption of the embryos and, also, the survival rate of the first litters was very low. Those animals surviving were undersized. Even with the addition of 6 micrograms of biotin the first generation offspring was small at weaning age. No resorptions occurred in the group so supplemented, however.

There was no improvement in the reproductive performance of the females which were fed 15 per cent casein to replace an equivalent amount of egg white in the diet. This poor performance was attributed to the lack of biotin, but it is also noted that the diets contained no "folic acid."

It was concluded that biotin is needed early in intrauterine life in the rat and, further, that it is necessary for successful lactation in this species. This report adds more evidence for a high biotin requirement of rapidly growing tissues such as the embryo and certain tumors (*Nutrition Reviews* **2**, *345* (*1944*)). The function of this vitamin, however, has not been defined.

THE FUNCTIONS OF PYRIDOXINE-LIKE COMPOUNDS

It is generally accepted that experimentally produced pyridoxine deficiency in rats may be manifested by a dermatitis, in dogs and swine by a microcytic anemia, and in all three species by neurologic lesions resulting in convulsions in dogs and swine (Lepkovsky, in Evans', "The Biological Action of the Vitamins," *p. 111, Univ. of Chicago Press, Chicago* (*1942*)). Further, pyridoxine deficient rats, dogs, and pigs have been shown to exhibit *in vivo* a defect in the metabolism of the amino acid tryptophane (*Nutrition Reviews* **3**, *72* (*1945*)). The manifestations of pyridoxine deficiency in man have not been clearly defined (*Ibid.* **2**, *182* (*1944*)).

Another relationship of pyridoxine to amino acid metabolism has been demonstrated by the finding that the decarboxylation of tyrosine by Streptococcus faecalis (S. lactis R) is dependent upon the presence in the medium of pyridoxine or pyridoxal (a derivative of pyridoxine in which the 4-methoxy group has been oxidized to an aldehyde) (*Nutrition Reviews* **3**, *63* (*1945*)) which had been phosphorylated (*Ibid.* **3**, *72* (*1945*)). Thus it seemed probable that pyridoxine or pyridoxal serves as a part of the coenzyme involved in the decarboxylation of tyrosine by bacteria. More recent studies support and extend this view.

Umbreit, Bellamy, and Gunsalus (*Arch. Biochem.* **7**, *185* (*1945*)) have described the experimental details and elaborated on some of the previously mentioned experimental findings of the role of pyridoxine compounds as coenzymes. They grew S. faecalis cells on a defined medium containing no pyridoxine but, instead, containing alanine which permitted the growth of these cells without the simultaneous production of codecarboxylase. The cells were harvested,

washed, dried *in vacuo*, and stored. The preparation could not decarboxylate tyrosine at a rapid rate until a source of codecarboxylase was added—it thus served as a test preparation of the apoenzyme essentially free of the coenzyme. A purified preparation of the natural coenzyme was obtained from yeast by a method of extraction and precipitation described by Gale and Epps (*Biochem. J.* **38,** *250 (1944)*). Pyridoxal was converted to an active codecarboxylase by two methods: (a) incubating it with the previously described apoenzyme preparation plus ATP (adenosine triphosphate), and (b) by chemical treatment of pyridoxal with the phosphorylating agent, phosphorus oxychloride. The structure of the resulting active material, presumably a phosphorylated pyridoxal, was not determined. Standard Warburg methods were employed in estimating the decarboxylation by determination of the rate of evolution of carbon dioxide.

It was demonstrated that pyridoxine, pyridoxamine, and 4-pyridoxic acid (a metabolite of pyridoxine in man) (Huff and Perlzweig, *J. Biol. Chem.* **155,** *345 (1944)*) were inactive as codecarboxylases whether incubated with the apoenzyme with or without ATP. The purified natural coenzyme and the synthetic coenzyme prepared from pyridoxal were both as active before incubation with ATP as after such treatment. Pyridoxal, which had not been phosphorylated, however, was active only after incubation with ATP. It seems reasonable, therefore, that pyridoxine must be oxidized to pyridoxal which, in turn, is phosphorylated in order to carry out its function as tyrosine decarboxylase in this organism. Umbreit and co-workers further imply the identity of this codecarboxylase with that coenzyme studied by Gale and Epps (*loc. cit.*) which also functions as a lysine decarboxylase (Gale and Epps, *Biochem. J.* **38,** *232 (1944)*).

In a later paper Umbreit and Gunsalus (*J. Biol. Chem.* **159,** *333 (1945)*) have prepared from E. coli a cell free decarboxylase preparation capable of decarboxylating arginine and glutamic acid. These enzymes were resolved to yield the corresponding apoenzymes by a process of aging and dialysis. As in the case of tyrosine decarboxylase, it was found that the addition of either the natural or synthetic (from pyridoxal) codecarboxylase to the apoenzyme preparation resulted in the decarboxylation of arginine or glutamic acid. Furthermore, pyridoxal itself again became active when incubated with ATP plus the enzyme preparation. Finally, the natural coenzyme of the arginine system was active as the coenzyme for a tyrosine system. The authors suggest that the vitamin B_6 group may act as the general coenzyme of amino acid decarboxylases. It is of interest to recall that the thiamine-protein enzymes serve a similar function with certain alpha-keto acids (see Green, "Mechanisms of Biological Oxidations," *p. 120, Cambridge University Press, London (1941)*).

Cohen and Lichstein (*J. Biol. Chem.* **159,** *367 (1945)*) have verified the findings of the Cornell workers that the activity of tyrosine decarboxylase of S. lactis R (S. faecalis) varies with the pyridoxine level of the growth media. However, they observe that this does not constitute proof that pyridoxine or a related compound enters into the formation of codecarboxylase. They point to the fact that Gale and Epps failed to demonstrate phosphorus in their co-factor while the coenzyme of Gunsalus *et al.* seems to be phosphorylated. This discrepancy has been frequently noted by the Cornell group and, although it remains to be explained, the evidence at the moment is strongly in favor of phosphorylated pyridoxal acting as a coenzyme for several amino acid decarboxylases.

This centering of interest on pyridoxal by these and other *in vitro* studies makes timely a report by Miller and Baumann (*J. Biol. Chem.* **159,** *173 (1945)*) dealing with the relative effectiveness of several vitamin B_6

substances for the growth of mice and for their effect on xanthurenic acid excretion. Weanling albino mice were depleted of vitamin B_6 by feeding a purified diet containing 10 per cent casein. The animals were then given a pyridoxine free diet containing 45 per cent casein. The weight changes and the xanthurenic acid excretion were observed after the separate feeding of the several pyridoxine derivatives in equimolar amounts. Pyridoxine hydrochloride returned the xanthurenic acid excretion to normal within four days, pyridoxal required seventeen days, and pyridoxamine twenty-four days. Similarly, the pyridoxine fed mice resumed growth more rapidly than did the pyridoxal fed animals. Those receiving pyridoxamine grew poorly throughout the experiment. At low levels the longest survival occurred with the pyridoxine fed mice; the shortest with those given pyridoxamine. These differences were not due to differences in the stability of the various materials in the diet. Similarly, it was found that pyridoxine promoted greater growth of rats fed a pyridoxine deficient diet than did the other two derivatives. Under the conditions employed by Snell and Rannefeld (*Ibid.* **157,** *475 (1945)*) these three compounds exerted similar activities on the growth of the rat. It is clear that pyridoxal is active for the growth of the rat and mouse as well as for reducing the excretion of xanthurenic acid by the latter species, although under certain conditions it may be less so than pyridoxine.

Miller and Baumann also investigated the metabolic defect which results in the urinary excretion of xanthurenic acid following tryptophane administration to the B_6 deficient animal. The expected influence of pyridoxine on xanthurenic acid excretion by the mouse after injection of tryptophane was verified. Xanthurenic acid was then fed to mice receiving no vitamin B_6 and to others, the diet of which was supplemented with pyridoxine. No differences in the excretion of xanthurenic acid by the two groups was observed. These facts were interpreted to indicate that xanthurenic acid may not be a normal metabolite of tryptophane and that "one function of pyridoxine is to regulate the metabolism of tryptophane by a route that prevents the formation of appreciable amounts of xanthurenic acid."

Much remains to be learned concerning the metabolic role of pyridoxine. However, it is apparent that this compound or its derivatives is intimately involved in protein metabolism. It should not be forgotten, however, that there is also evidence to indicate that vitamin B_6 may be related to the metabolism of certain fatty acids, a relationship which has never been completely clarified.

RELATION OF NUTRITIONAL DEFICIENCY TO VIRULENCE OF AVIAN MALARIA

In a previous review (*Nutrition Reviews* **1,** *362 (1943)*) the relationship of nutritional deficiency and malarial infection was discussed. Trager (*J. Exp. Med.* **77,** *557 (1943)*) found that biotin deficiency increases the severity of Plasmodium lophurae infection in birds. Seeler and Ott (*J. Infect. Dis.* **75,** *175 (1944)*), on the other hand, found that riboflavin deficiency reduces markedly the severity of this infection. In another communication (*Ibid.* **77,** *82 (1945)*) the latter workers report on the influence of "folic acid" deficiency and deficiency of other unidentified factors on this infection.

Day-old male single comb White Leghorn chicks were kept on Startena, a commercial stock ration, for four days, after which time two equivalent groups were selected for uniform weight. One of these was given a deficient diet and the other was continued

on Startena or was given the deficient diet supplemented with 15 per cent dried beef liver in the place of an equal amount of dextrose. The deficient diet contained the fat soluble vitamins as well as thiamine, riboflavin, niacin, pyridoxine, choline, p-aminobenzoic acid, inositol, and biotin. On the eleventh day of age the chicks were inoculated with P. lophurae and the course of the infection was followed by noting the number of erythrocytes infected with parasites on the fifth, seventh, and ninth days after inoculation. The strain of P. lophurae introduced into the deficient chicks was transferred at weekly intervals through successive groups of deficient chicks for a total of sixteen weeks. The infection was introduced and transferred in a similar manner in chicks fed the adequate ration.

The initial injection of parasites in the deficient animals produced a distinctly higher parasite count than in the chicks given an adequate diet. Thus, seven days after inoculation 16.1 per cent of the red cells of the former group were parasitized whereas only 4.1 per cent of the red cells of the chicks receiving the adequate diet were so affected. Continued passage of the parasite through the chicks did not increase the severity of the infection beyond that reached in the initial injection and transfer to other hosts receiving a deficient diet.

In order to determine whether the greater severity of the infection in the deficient birds represented a change in the virulence of the parasite or was due to the environment within the host, a series of transfers were made from the deficient chicks to chicks receiving adequate diets. Significantly, the latter showed parasite counts comparable to those of well fed chicks originally infected. Apparently, the severity of the infection was directly related to the nutrition of the host.

INFLUENCE OF AMINO ACID MIXTURES ON PLASMA PROTEIN PRODUCTION

The availability of various amino acids in pure form and the technic of plasmapheresis make possible a study of the role of amino acids in plasma protein production. When blood is withdrawn and the red cells suspended in saline instead of their original plasma are returned to the animals, depletion of the normal levels of plasma proteins in circulating blood is effected and a strain is put upon the body which accentuates the normal demand for plasma protein production. This probably stimulates vigorous new plasma protein production, at times, to the point of diversion of protein building materials from the tissues.

Madden, Anderson, Donovan, and Whipple (*J. Exp. Med.* **82,** 77 (*1945*)) present the results of studies which sought to determine the influence of individual amino acids on plasma protein production. The method of study was similar to that in experiments from their laboratory which have been described on a number of occasions in this journal. Amino acids were given subcutaneously or intravenously as infusions containing concentrations of 6 to 10 per cent in distilled water. In certain instances these were also given orally. The experiments were not designed to study vitamin deficiencies and the synthetic diets necessary to the investigation provided certain unanticipated dietary deficiencies. In the animals studied, certain skin lesions were observed and some impairment of liver function occurred, as indicated by reduced excretion of bilirubin. These are regarded by the investigators as not being the result of amino acid inadequacies or imbalance. Failure to foresee the possible development of vitamin deficiency as a complication of their experiment probably does not invalidate the observations made and the conclusions reached.

It was found that when the diet nitrogen was provided by certain mixtures of the ten

amino acids "essential" for growth in the rat plus glycine, given intravenously at a rapid rate, plasma protein production in dogs was good. When the same mixture was given subcutaneously at a slower rate it seemed to be somewhat better utilized. It is of interest as well as of practical importance to note that the same amino acids fed orally were definitely better utilized and were associated with a lower urinary nitrogen excretion as compared with the same amino acids given parenterally.

The addition of amino acids other than the ten "essential" ones did not appear to speed up production of plasma protein. On the other hand, the omission of certain essential amino acids had a pronounced effect. The interpretation of the specific influence of withdrawal of a single amino acid upon new plasma protein synthesis is, of course, difficult. When an amino acid essential for total body protein maintenance is lacking in the diet internal shifts of protein occur which may, at least temporarily, obscure certain of the effects. In an earlier report Madden and co-workers (*J. Exp. Med.* **77**, *277* (*1943*)) found that when threonine or valine was omitted the decline of plasma protein production was sharp within the first week and urinary nitrogen was high. These effects were noted even though little weight change occurred. In the more recent experiments, when leucine and isoleucine were omitted the decline in plasma protein production was slow, running into the third week, but urinary nitrogen promptly exceeded the nitrogen intake and weight loss was sharp. When only isoleucine was returned to the intake excellent plasma protein regeneration took place although high urinary nitrogen and weight loss remained as evidences of leucine deficiency. The investigators suggest that this may represent "raiding" of body proteins for the leucine needed in plasma protein regeneration. They observed a definite plasma icterus. They, therefore, raise the question as to whether increased red blood cell destruction with hemoglobin splitting may have provided the leucine required for new plasma protein production. Further study of this question is obviously necessary before any conclusion can be drawn.

The omission of lysine, histidine, and arginine as a group was associated with a prompt depression of plasma protein production although nitrogen balance was positive despite some increase in urinary output. The omission of tryptophane was associated rather promptly with reduced plasma protein formation. When methionine was omitted and cystine given, plasma protein production was excellent although the urinary nitrogen increased considerably and weight loss occurred.

It was observed that during periods of an essential amino acid deficiency the output of plasma protein was sometimes sustained for one, two, or even three weeks. It seems probable, therefore, that the body draws on some source supply of the missing amino acid without which prolonged production of new plasma protein is impossible. This raises the question as to whether certain amino acids may be withdrawn from appropriate tissue protein without destruction of such protein. It may also be asked whether amino acids lost from normal body tissue wear and tear or from incidentally accelerated catabolism can be recaptured. Whether protein may be actually destroyed to supply the deficient amino acids is, in the opinion of the investigators, an open question.

Consistent with these observations, it was noted that after a deficiency period, when the missing amino acid was returned to the mixture or even when a complete protein was fed, some delay in the output of new plasma protein was observed. This sometimes was as long as two or three weeks. Perhaps in an emergency the tissue cells have a definite priority over plasma protein accumulation until the urgent deficiency is made up.

In view of the limitations of these experiments which the authors themselves point

out, the results obtained can be regarded only as suggestive rather than conclusive. Nevertheless, they are of great interest and represent another move in the direction of a more complete understanding of protein metabolism.

ANEMIA IN SCURVY

When persons who have subsisted on a diet grossly deficient in many vitamins and other factors are hospitalized and fed a nutritious diet, it is unsafe to ascribe improvement of a hyperchromic macrocytic anemia to therapy with ascorbic acid, even though it effects dramatic relief of concomitant scurvy. In an article on high-color-index anemia due to ascorbic acid deficiency, Gottlieb (*Brit. Med. J.* **2,** *119* (*1945*)) described 4 cases of "bachelor scurvy" in males, aged 70 to 80 years, who lived alone and had subsisted chiefly on bread, margarine, and cheese. One of the patients died of heart failure shortly after admission. The response of the other 3 to large doses of ascorbic acid was characteristic of its specific effects on scurvy. Two of the 3 who were treated had normal amounts of free hydrochloric acid in the gastric juice. In the absence of a control diet during the period of treatment with ascorbic acid of the normocytic, normochromic anemia in adults with scurvy (*Nutrition Reviews* **3,** *178* (*1945*)), it is impossible to evaluate the possible role of extrinsic factor or other dietary constituents. Furthermore, the anemia resembles much more closely that seen in patients whose diet contained little extrinsic factor and whose anemia responded to extrinsic factor or liver extract (*Ibid.* **3,** *154* (*1945*)). A relationship of ascorbic acid to pernicious anemia described by Dyke, Della Vida, and Delikat (*Lancet* **II,** *278* (*1942*)) has not been studied extensively and the mechanisms involved have not been investigated. In spite of the very suggestive testimony of Gottlieb's report the absence of rigid controls in diet during the period of treatment with ascorbic acid leaves the results speculative. Ascorbic acid metabolism in refractory macrocytic anemias requires further study and the anemia of scurvy may furnish information as to the role of ascorbic acid in hematopoiesis. At present there is too little factual data by which to appraise such relationships.

POLICIES OF THE COUNCIL ON FOODS AND NUTRITION OF THE AMERICAN MEDICAL ASSOCIATION REGARDING NUTRITIVE QUALITY OF FOODS

The Council on Foods and Nutrition has long concerned itself with the quality of foods. At first this interest involved primarily the purity of individual products and the truthfulness of statements made on package labels and in advertising. More recently the attention of the Council has also been given to the nutritional quality of foods and to the effect of a poor nutritional environment on the public health. A recognition of the importance of natural foods of both plant and animal origin in the maintenance of high standards of nutrition is paramount. It is necessary that the vast bulk of our diets in the way of carbohydrates, fats and proteins must come from products of this type. While certain nutrients found in these foods may be prepared synthetically, nutritional knowledge is incomplete, and unknown food factors undoubtedly exist which can be obtained only from natural food products.

It is well known, however, that many foods have suffered deterioration in their

nutritional values as a result of the influences of modern civilization. Fruits and vegetables have been influenced by selective breeding to give better yields per acre and to have better keeping qualities in market or a better appearance. These induced changes have frequently brought about a reduction in the content of vitamins or minerals, or both, such that consumption of natural foods does not always insure adequate nutrition. The preference of the public for processed foods has frequently caused many of the valuable nutrients of a food to be discarded in favor of the nutritionally inferior, smooth, bland parts of the natural food. The use of high temperatures in the preparation of certain types of foods further reduces their value to the body. Examples of these effects may readily be found in the chemically pure sugar of commerce, which lacks all but traces of its original vitamins and minerals, in white flour, from which the nutritious germ and outer coatings have been removed, and in the refined cereals, which undergo various processing procedures. These "denatured" foods have come to replace in large part the nutritious more nearly whole cereal grains which in former days supplied a considerable portion of the vitamins of the B group that enter the diet.

A consequence of this sophistication of the modern food supply has been to increase the difficulty of selecting diets that are suitable to maintain a good state of nutrition. Extensive educational programs have failed to bring about a greater appreciation and usage of the natural type of foods, such as whole wheat flour and bread. The continuation and extension of the preference for nutritionally inferior foods poses a question of grave concern with respect to the effect on the public health. This is especially disturbing, since animal experiments have shown that ill effects resulting from less than satisfactory diets may become obvious only after many years.

In view of these facts the Council wishes to encourage efforts to improve as far as possible the nutritive quality of all foods which contribute importantly to the American diet and which thereby constitute the food environment of the people. A genuine interest is taken by the Council in all attempts to raise the level of nutrients in products of the farm and in the institution of measures designed to retain in high degree the nutrients of foods during storage, processing and cooking. Programs of the dairy industry directed toward the production of milk of a higher and more uniform vitamin A content have had the support of the Council. Recently plans have been made to grant acceptance and use of the Council Seal to canned tomato and citrus juices, which contain a high natural content of vitamin C. The Council has also encouraged limiting consumption of sugar to such amounts as are needed to increase the palatability of foods with significant nutritional values.[1]

In spite of all these efforts there exist now and will undoubtedly continue to exist for some time certain specific deficiencies in large segments of the population which can be remedied best through addition of the indicated nutrients to cheap, staple foods that occupy substantial places in the dietary.[2] The Council has favored and encouraged the addition of certain nutrients to selected foods for the purpose of overcoming these deficiencies by replacing as nearly as possible that which has been lost in processing or by making appropriate foods serve as carriers of dietary essentials which otherwise are inadequately supplied by many diets. Such additions considered to be in the inter-

[1] Council on Foods and Nutrition: Some Nutritional Aspects of Sugar, Candy and Sweetened Carbonated Beverages, J. A. M. A. **120**: 763 (Nov. 7) 1942.

[2] This statement of principle respecting addition of nutrients to foods is in harmony with the policy of the Federal Security Agency as promulgated July 3, 1943, Federal Register, July 3, 1943, p. 9170.

est of the public health are enumerated as follows:

Restoration (referred to as enriched in instances in which federal standards have been enacted)

of processed cereals with thiamine, riboflavin, niacin and iron.

of corn meal with thiamine, niacin and iron.

of hulled rice with thiamine, niacin and iron.

Enrichment of white flour with thiamine, riboflavin, niacin and iron.

Enrichment of white bread and rolls with thiamine, riboflavin, niacin and iron.

Enrichment of farina with thiamine, riboflavin, niacin and iron.

Fortification

of milk with 400 units of vitamin D per quart.

of oleomargarine with not less than 9,000 U. S. P. units of vitamin A per pound.

of salt with iodine.

The Council reaffirms its expression of opinion respecting the conditions under which additions of vitamins or minerals to foods will be approved as stated here and previously.[3] As more knowledge is acquired, some of the present "enrichment" programs may be modified. Some may be discontinued and new ones may be added. It is believed, however, that the present guiding principles will be maintained. In any case the addition of synthetics to natural foods must depend on the prevention of known deficiencies in groups of the population and not on the utilization of synthetic products which happen to be available at low cost. In the future the synthetics may be used more efficiently in animal nutrition to decrease the cost of animal products so that these materials may be more widely used in the human diet.

The Council disapproves of unlimited or indiscriminate fortification of general purpose foods with minerals, vitamins, amino acids or other nutrients. It likewise disapproves of artificial combinations of edibles designed to carry vitamins, minerals, amino acids or other nutrients for the use of the general public. It will use its influence to discourage the sale of products of the latter type except as special purpose products appropriately labeled to indicate their special purpose uses. (Reprinted from the *J. Am. Med. Assn.* **129,** *348 (1945.)*)

[3] Annual Meeting of the Council on Foods, J. A. M. A. **123:** 681 (Aug. 19) 1939.

NOTES

Letter to the Editor

Dear Sir:

The writer recognizes that it is a difficult matter to review a complex subject in a short space and in limited time, but the recent article "Folic Acid and Related Factors" (*Nutrition Reviews* **3,** *165* (*1945*)) contains inaccuracies which should not go uncorrected. Perhaps it is not the intention of the editors to include exhaustive reviews in this journal (It is not.—Ed.) but if they permit the use of general titles such as the one referred to, it would seem that editorial policy should insist on a corresponding degree of comprehensiveness in the reviews.

In discussing the possible relationship of xanthopterin to the various factors the reviewer states "After Wright and Welch... xanthopterin was tested in a number of laboratories for its effect on experimental animals." It should be pointed out that such tests were carried out in milk-anemic rats (Tschesche and Wolf, *Z. f. physiol. Chem.* **248,** *34* (*1937*)), anemic fish (Simmons and Norris, *J. Biol. Chem.* **140,** *679* (*1941*)), cytopenic monkeys (Totter *et al.*, *Federation Proceedings* **2,** *72* (*1943*)), sulfa treated rats (Totter and Day, *J. Biol. Chem.* **147,** *257* (*1943*)), and in chicks (O'Dell and Hogan, *J. Biol. Chem.* **149,** *323* (*1943*)) before the publication of Wright and Welch (*Science* **98,** *179* (*1943*)).

The review in question cites a paper by Mallory, Mims, Totter, and Day (*J. Biol. Chem.* **156,** *317* (*1944*)) in connection with work on xanthopterin. The publication referred to offers no evidence of any kind concerning the effect of xanthopterin on experimental animals. The authors of the paper, contrary to the statement by the reviewer, did not and do not now "agree" that xanthopterin has "little or no activity" in the monkey. As a matter of fact in the last publication from the same laboratory dealing with the pterin, the statement was made that xanthopterin was found to be invariably active when fed to cytopenic monkeys although it did not permanently prevent vitamin M deficiency (Totter, Mims, and Day, *Science* **100,** *223* (*1944*)).

In summarizing the activities of the various factors the review refers to Stokstad's L. casei factor as being active only for L. casei. This factor, when its isolation was announced, was stated to be active for the chick (Hutchings, Stokstad, Bohonos, and Slobodkin, *Science* **99,** *371* (*1944*)). It is also active for the sulfa treated rat (Daft and Sebrell, *Pub. Health Reports* **58,** *1542* (*1943*)), highly active for the vitamin M deficiency monkey, and extremely potent in stimulating growth of S. lactis R after enzymic treatment (Day *et al.*, *J. Biol. Chem.* **157,** *423* (*1945*)).

In reviewing a subject as complex as the one covered in the article under discussion, it would be understandable for the reviewer to make errors in interpretation; errors of fact can be less readily excused, however. Nutrition Reviews has served a useful and unique function in its field, and should continue to do so. It would be regrettable to have its usefulness impaired by inexact reviewing.

Sincerely,

John R. Totter,
Department of Physiological Chemistry,
University of Arkansas School of Medicine,
Little Rock, Arkansas.

Nutritional Problems In Mass Feeding

McCay (*Am. J. Pub. Health* **35,** *143* (*1945*)) has surveyed the subject of nutritional problems in mass feeding in the light of experience gained from his extensive studies of navy kitchens and mess halls. Perhaps the most seriously affected nutrient

in mass cooking is ascorbic acid, because of the ease with which it is oxidized in solution when extracted from plant cells. The problem of preserving this vitamin has been attacked in a number of ways, such as improved methods of drying foods, decreasing the cooking time of vegetables, and preparing salads from fruits and vegetables cut into large pieces instead of shreds.

An interesting suggestion is made by McCay about the use of citrus fruit peels. He points out that these might be used instead of being put into the garbage, since they contain large amounts of ascorbic acid which could be largely preserved by preparing marmalade in individual kitchens with suitable precautions. Other advantages following the salvage of such peelings in the forms of marmalade would be to improve the attractiveness of meals, increase the consumption of bread, and satisfy the longing for sugar by making large quantities of marmalade available.

Bread is used less then formerly, but is still an important item of diet, and McCay suggests that more might be consumed if more varieties were used and attractive spreads provided. The nutritional quality of bread, though decreased by overmilling of the wheat, has in recent years been improved by enrichment and by the use of milk solids in preparing the dough.

In the mass feeding of navy men in this country it was observed that they generally eat about 12 per cent of their calories outside the regular mess hall, and it is suggested by McCay that the candy bars and pastries purchased be improved from the nutritional point of view. It is well known that poor cookery and the serving of strange foods will lead to heavy wastage, particularly of foods planned by the dietitian for their vitamin content, and this can be prevented only by a suitable educational campaign.

The beverages provided in mass feeding are becoming more and more important in supplying certain of the nutritional essentials, largely because of the availability and content, and this can be prevented only by a consumption of milk and fruit juices. Additional reasons are that beverages are simply prepared, quickly consumed, and leave little waste.

Electronics and Nutrition

On first consideration, the rapidly expanding field of electronics would appear to bear little relation to nutrition. However, the recent report of Moyer and Stotz (*Science* **102,** *68 (1945)*) indicates that the blanching of vegetables by electronic heat points the way to production of processed fruits and vegetables of higher nutritive value.

It is generally recognized that a preliminary heat treatment of raw vegetables to destroy enzymes is required to prevent deterioration of flavor, color, and certain vitamins during storage in the frozen or dehydrated state. The commonly used boiling water or steam blanching often damages the desired texture of the product and leaches out considerable of the soluble vitamins. The internal heating provided by electronic power cooks a vegetable, so to speak, "in its own juice." In their preliminary report these investigators have demonstrated that the 30 to 40 per cent loss of ascorbic acid encountered during steam and water blanching of shredded cabbage was reduced to a negligible 3 per cent by electronic blanching. Further studies with other products are promised.

Nutrition and Industrial Poisonings

The Food and Nutrition Board of the National Research Council has adopted a resolution stating that there is real need for systematic investigation of the role of nutrition in the prophylaxis and therapy of industrial poisonings. Two general lines of experimentation are indicated: (1) on the protective and therapeutic value of good over-all nutrition for the exposed worker; (2) on the value of specific nutrients in the prophylaxis and treatment of specific poisonings. In addition to fundamental types of investigation, the Board wishes to stress the need for work bearing a close relation to problems occurring in industry.

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The Nutrition Foundation was organized by manufacturers of the food industry to carry out a program of public service.

The purposes of the Foundation are:

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2. The development of an educational program to facilitate the widest possible usage of what is known in the science of nutrition.

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The Editorial Staff has been carefully selected so that the publication will merit in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Assistant Editors.

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